EFFECTS OF X-IRRADIATION UPON POSTNATAL GROWTH IN THE MOUSE[1]

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Relatively little attention has been directed towards the long-term effects of embryonic or fetal irradiation on the subsequent vigor and well being which the irradiated may attain in later life. However, since an individual's development, in a sense, unfolds from conception until death, it was felt desirable to investigate systematically effects of *in utero* irradiation upon postnatal development. The present study has emphasized effects on development after parturition in mice, as measured by such responses as growth from birth to maturity, lifetime fecundity, and total lifespan, of which the growth effects will be reported in this paper.

The mammalian embryo is in a unique stage of development because of the great number of cells that are actively undergoing differentiation. Radiant energy absorbed during a period of development may act as an agent directing the organism's development into new paths. The redirection may stimulate either nuclear changes which have permanent continuity in later cell generations or cytoplasmic influences which may possibly be more transient. Of the various agents which may make these changes, ionizing radiations are especially useful since their penetrant actions have a general distribution throughout the entire organism. Patterns of sensitivity which are characteristic of the embryo may be revealed, therefore, by the selective response of the exposed structures.

Numerous congenital malformations have been reported as a result of embryonic radiation. Most of the earlier works are difficult to interpret since careful control of the embryo's age at irradiation was not made and the physical factors of radiation often were not standardized. These changes involved many organs of the developing individual. They indicate well defined critical periods during which the cells are susceptible to redirection of development. They further indicate that the susceptibilities change as development progresses, making some elements more resistant and causing other elements to enter more sensitive periods. In general for any one type sensitivity the periods appear to be quite restricted in the length of time over which they are active. There is furthermore a dose-dependence. Measurement of these effects has largely been confined to the observations made on the young prior to parturition or but a few days thereafter. Long-term effects have scarcely been considered.

An additional major shortcoming of many of the earlier experiments is the fact that the animals used were either animals of unknown heterogeneous origin

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or else animals all of uniform origin. In this study several genetic backgrounds were utilized in order to obtain information on important genotypic-environmental interactions. The use of several different genotypes makes it possible to draw more valid inferences from a single experiment to an over-all population of animals than if the results were gained from a single genetic background. The effects of *in utero* irradiation upon postnatal development in this study have been measured in three inbred strains of mice and all their possible hybrids.

Materials and Methods

The mice used in this investigation were taken from three inbred strains maintained at the Genetics Laboratory of Iowa State University. The strains were designated by the Committee on Mouse Nomenclature as BALB/Gw, K, and S. The strains were differentiated originally by resistance to mouse typhoid (Gowen, 1948), but are known to differ in a number of other physiological characters, including differences in response to various effects of irradiation (Grahn, 1954; Gowen and Stadler, 1956; Stadler and Gowen, 1957a, 1957b). The three strains cover a wide range of the spectrum of radiation response, the Ba and K strains representing relatively susceptible strains and the S strain representing a relatively resistant strain.

Animals that were to be irradiated as embryos were obtained exclusively from first litters of matings within and among the three inbred strains, including reciprocal matings. Females were examined daily for the presence of a vaginal plug, this plug being the sole criterion used to time the period of gestation and the approximate age of embryos at irradiation. Although the majority of matings in mice take place during the night, they have been known to occur throughout the entire 24-hour period. In this experiment, nevertheless, all mice were considered to have mated during the same time of night. The time of 4:00 AM was chosen as the approximate time of fertilization since Snell (1940) determined that in the Bagg strain of mice the modal ovulation period was between midnight and 3:00 AM, with fertilization occurring shortly after liberation of the egg. All pregnant females were irradiated at about 4:00 PM so that the embryological ages at irradiation were approximately $6\frac{1}{2}$, $10\frac{1}{2}$, $14\frac{1}{2}$, and $17\frac{1}{2}$ days. These embryological ages were chosen since they represent, in the mouse, developmental stages which correspond to a period shortly after implantation, a period during the height of major organ formation, a period of minor organ formation, and a period concerned with growth of the fetus.

The experiment was designed as a factorial with three strains of mice and all their possible hybrids, making a total of nine different inheritance types, and five levels of irradiation including 0, 20, 80, 160, and 320 roentgens.

Pregnant females were examined in the morning and in the evening, so that newborn litters usually were found within 12 hours of birth. Mice in a litter were marked individually at birth by means of india ink injected from a hypodermic needle subcutaneously. Individuals were weighed at birth, 12, 26, 40, 60, and 75 days of age. Birth weights were recorded to the nearest hundredth of a gram, and all other weights to the nearest tenth of a gram. All litters were weaned at 30 days, and the males and females separated at this time.

Within each dose-embryological age treatment a minimum of two males and

two females of each inheritance type was sought by 75 days. The experiment had unequal subclass numbers, due to differential litter sizes and differential postnatal viability. It was necessary, therefore, to use disproportionate frequency analyses of all the data because of the unequal subclass numbers. The statistical procedures used in the analysis of the data are essentially as those described by Snedecor (1956), and additional details will be explained with the presentation of results.

In addition to the irradiation of embryos, a second phase of the experiment was undertaken in order to determine effect of x-irradiation upon newborn litters. It was hoped that this aspect of the experiment would also help to elucidate some of the radiation effects that were due to direct effects on the embryos and other effects that may have been mediated through the maternal organism. Litters were irradiated at 4:00 PM on the day they were born. The dam did not receive any irradiation. The same strains of mice and the same levels of radiation were used in this study. Both experiments were conducted in a well-ventilated room, in which the environment and management were relatively constant. Food and water were provided *ad libitum*.

The source of irradiation was a General Electric Maxitron which operated at 250 pkv, 30 ma with 0.25 mm. Cu + 1 mm. Al filtration at a distance of 50 cm. from anode to mid-mouse. The dose rate was approximately 133 r/minute, the dosage rates having been measured in air by means of a rate meter. Pregnant mice were exposed to single doses of whole-body irradiation within a circular, wooden container, $6\frac{1}{2}$ inches in diameter, and 1 inch in depth. The base of the container was $\frac{1}{4}$- by $\frac{1}{4}$-inch wire mesh, and the top was covered with two layers of cellophane. In the experiment in which newborn litters were irradiated, the entire litter was exposed to whole-body irradiation in small, plastic trays, and then immediately returned to their dam.

A complete description of the methods and results of this investigation may be found in a doctoral dissertation (Nash, 1960) on file at the Iowa State University Library.

Results

Irradiation of mouse embryos

In the results that follow the two sexes are treated separately, in accordance with the generally observed fact that male mice grow more rapidly than females. In interpreting the data it was also necessary to take into account the fact that growth in the mouse is known to be affected by litter size. Individuals from smaller litters tend to grow faster than individuals from larger litters. The body weight data in this study supported this conclusion. The effect of litter size or weight was not of primary interest in this experiment, and also added an unnecessary complication in the interpretation of the main effects. This variable could not be controlled experimentally but could at least be standardized by a statistical adjustment of the data. Consequently all weights from birth to 75 days utilized in the analysis were adjusted for litter size at birth. A litter size of nine, which represents the mean over-all treatments, was used as the base point in adjusting for litter size. The regressions of body weight on litter size at birth were calculated for each treatment. Some heterogeneity between regression coefficients of the

different treatments was noted. However, it seemed clear that the use of the mean regression coefficient of all treatments for adjusting the body weights would be the most adequate means of standardizing the data.

Radiation response of males

The body weight means and standard errors for the male progeny are shown in Table I. It is evident that body weight response to *in utero* irradiation is

TABLE I

Body weight means and standard errors for male progeny; all weights adjusted for litter size at birth

Embryological age at irradiation	Irradiation dose	Age postparturition in days					
		Birth	12	26	40	60	75
Control	0 r	1.40 ± .02	5.0 ± .1	8.9 ± .4	17.7 ± .4	23.1 ± .3	24.7 ± .3
6½ days	20 r	1.36 ± .02	5.3 ± .2	9.7 ± .4	19.1 ± .4	23.8 ± .4	25.9 ± .4
	80 r	1.36 ± .02	5.5 ± .2	10.2 ± .4	19.2 ± .5	24.1 ± .3	25.9 ± .3
	160 r	1.35 ± .02	6.1 ± .2	11.3 ± .3	20.1 ± .5	24.6 ± .4	26.7 ± .4
10½ days	20 r	1.39 ± .02	5.5 ± .1	10.8 ± .3	20.1 ± .3	24.6 ± .3	25.9 ± .4
	80 r	1.25 ± .01	5.1 ± .1	9.1 ± .3	18.1 ± .3	21.7 ± .3	23.0 ± .3
	160 r	0.97 ± .02	—	—	—	—	—
	320 r	0.61 ± .02	—	—	—	—	—
14½ days	20 r	1.37 ± .01	4.9 ± .1	9.3 ± .3	18.7 ± .3	23.4 ± .3	24.7 ± .3
	80 r	1.31 ± .02	4.9 ± .2	8.9 ± .3	17.4 ± .7	22.2 ± .6	23.6 ± .6
	160 r	1.25 ± .02	4.9 ± .1	7.3 ± .3	14.5 ± .4	19.7 ± .4	21.3 ± .3
	320 r	0.98 ± .02	4.1 ± .5	5.8 ± .6	7.6 ± 1.0	8.8 ± .1	9.1 ± .2
17½ days	20 r	1.39 ± .01	5.1 ± .1	9.2 ± .3	18.0 ± .6	23.7 ± .4	25.3 ± .4
	80 r	1.35 ± .02	5.0 ± .2	9.2 ± .6	17.5 ± .6	23.2 ± .4	24.6 ± .4
	160 r	1.33 ± .02	4.7 ± .1	8.4 ± .4	16.0 ± .6	21.0 ± .3	22.1 ± .3
	320 r	1.32 ± .02	4.1 ± .1	6.6 ± .3	12.2 ± .7	17.1 ± .6	18.6 ± .5
Newborn	0 r	1.35 ± .02	5.3 ± .1	9.5 ± .3	17.9 ± .4	23.7 ± .3	25.4 ± .3
	20 r	1.37 ± .01	4.7 ± .2	9.1 ± .4	17.9 ± .6	22.6 ± .4	24.1 ± .4
	80 r	1.36 ± .01	5.3 ± .1	9.3 ± .3	17.8 ± .4	22.1 ± .3	23.6 ± .3
	160 r	1.35 ± .01	4.7 ± .2	8.3 ± .4	15.3 ± .7	20.1 ± .8	21.7 ± .6
	320 r	1.37 ± .01	4.2 ± .2	6.6 ± .3	12.4 ± .5	16.8 ± .5	18.8 ± .5

dependent both on the level of irradiation and on the age of the embryo when irradiated. The effects of irradiation on postnatal viability will be presented in a future paper, but it is evident from Table I that there may be considerable differential postnatal mortality, as seen in the absence of any embryos that received 160 r or 320 r at 10½ days surviving to 12 days post-partum.

Irradiation at 6½ days

Irradiation at 6½ days with doses up to 160 r apparently had no deleterious effect on postnatal growth. However, no litters were born to any of nine females

that had been exposed to 320 r at this stage of pregnancy, indicating that this dose may cause a 100% prenatal loss of progeny. Embryos that had received 160 r even showed an accelerated growth compared to controls. A significant difference in body weights was observed by the twelfth day and continued through 75 days. The relative difference reached a maximum at 26 days (27%) and appeared to be leveling off at about 8% at 75 days.

TABLE II

Body weight means and standard errors for female progeny; all weights adjusted for litter size at birth

Embryological age at irradiation	Irradiation dose	Age postparturition in days					
		Birth	12	26	40	60	75
Control	0 r	1.32 ± .01	4.9 ± .1	8.0 ± .3	15.9 ± .4	19.9 ± .3	21.1 ± .3
6½ days	20 r	1.28 ± .03	5.4 ± .2	10.0 ± .4	16.9 ± .6	20.7 ± .3	21.9 ± .3
	80 r	1.35 ± .01	5.1 ± .1	8.9 ± .3	16.4 ± .3	20.0 ± .2	21.5 ± .2
	160 r	1.23 ± .02	5.8 ± .2	10.8 ± .4	17.9 ± .5	21.2 ± .5	21.6 ± .4
10½ days	20 r	1.33 ± .02	5.5 ± .1	10.1 ± .3	17.9 ± .3	20.9 ± .4	22.0 ± .3
	80 r	1.21 ± .01	5.3 ± .2	9.0 ± .3	15.8 ± .3	18.4 ± .4	19.6 ± .4
	160 r	0.97 ± .02	—	—	—	—	—
	320 r	0.57 ± .02	—	—	—	—	—
14½ days	20 r	1.32 ± .01	4.8 ± .1	8.7 ± .2	15.8 ± .2	19.0 ± .2	20.6 ± .2
	80 r	1.31 ± .02	4.9 ± .1	9.2 ± .2	16.5 ± .3	19.6 ± .3	20.5 ± .3
	160 r	1.15 ± .01	4.4 ± .1	6.3 ± .2	12.0 ± .3	15.5 ± .3	16.6 ± .3
	320 r	0.96 ± .01	5.5 ± .2	6.8 ± .1	8.9 ± .9	10.5 ± .6	12.0 ± .0
17½ days	20 r	1.36 ± .01	5.2 ± .1	9.7 ± .3	17.1 ± .3	20.2 ± .2	21.8 ± .2
	80 r	1.31 ± .02	5.2 ± .2	9.2 ± .4	16.4 ± .4	20.0 ± .3	21.0 ± .3
	160 r	1.30 ± .02	4.8 ± .1	8.4 ± .4	14.4 ± .4	17.8 ± .3	18.8 ± .3
	320 r	1.25 ± .01	3.9 ± .1	6.4 ± .3	10.5 ± .5	14.4 ± .5	15.7 ± .5
Newborn	0 r	1.30 ± .01	5.3 ± .1	9.5 ± .4	16.6 ± .4	19.9 ± .3	20.8 ± .4
	20 r	1.32 ± .01	4.8 ± .2	8.5 ± .3	15.9 ± .3	18.9 ± .3	19.6 ± .4
	80 r	1.33 ± .01	4.9 ± .1	9.1 ± .3	15.7 ± .3	18.4 ± .3	19.6 ± .3
	160 r	1.34 ± .01	4.8 ± .2	8.2 ± .4	14.5 ± .4	16.8 ± .3	17.8 ± .3
	320 r	1.32 ± .01	4.3 ± .1	6.5 ± .2	11.3 ± .4	14.2 ± .3	15.4 ± .3

Irradiation at 10½ days

Doses of 80 r and above had noticeable effects on growth when given at 10½ days, resulting in lowered body weights at birth. After a dose of 320 r birth weights were less than half of those of controls. Embryos exposed to 160 r or 320 r were stillborn or died within a few days of birth. Although mice that had been exposed to 80 r weighed less than controls at birth, the survivors could not be distinguished from controls again until 60 days after birth. By 75 days the difference amounted to 7%.

Irradiation at 14½ days

Differences in birth weights were apparent after irradiation with 160 or 320 r. However, by the twelfth day of observation all irradiated groups were indistinguishable from controls. By 26 days of age animals that had received 160 r or

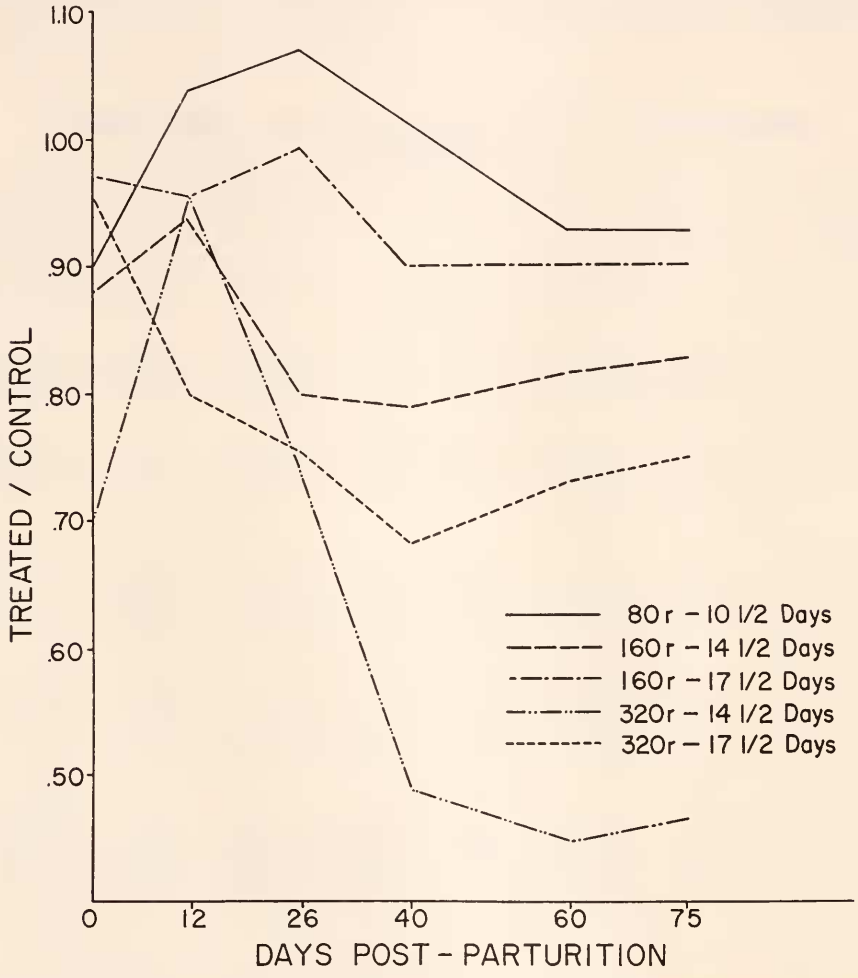

FIGURE 1. Ratio of the treated body weight means to control body weight means.

320 r were significantly lighter than controls. This pattern held throughout the rest of the period of observation, 160 r progeny weighing 89%, and 320 r progeny only 37% of controls at 75 days.

Irradiation at 17½ days

Following irradiation at 17½ days there was no significant effect on birth weights. By 12 days 320 r progeny had a lower weight than controls, and remained

lower, reaching a minimum at 40 days when they were only 68% of control weights, and recovering to only 75% by 75 days. A difference between 160 r progeny and controls became evident at 60 days and continued to 75 days. Doses of 20 r or 80 r apparently did not produce significant changes at any period.

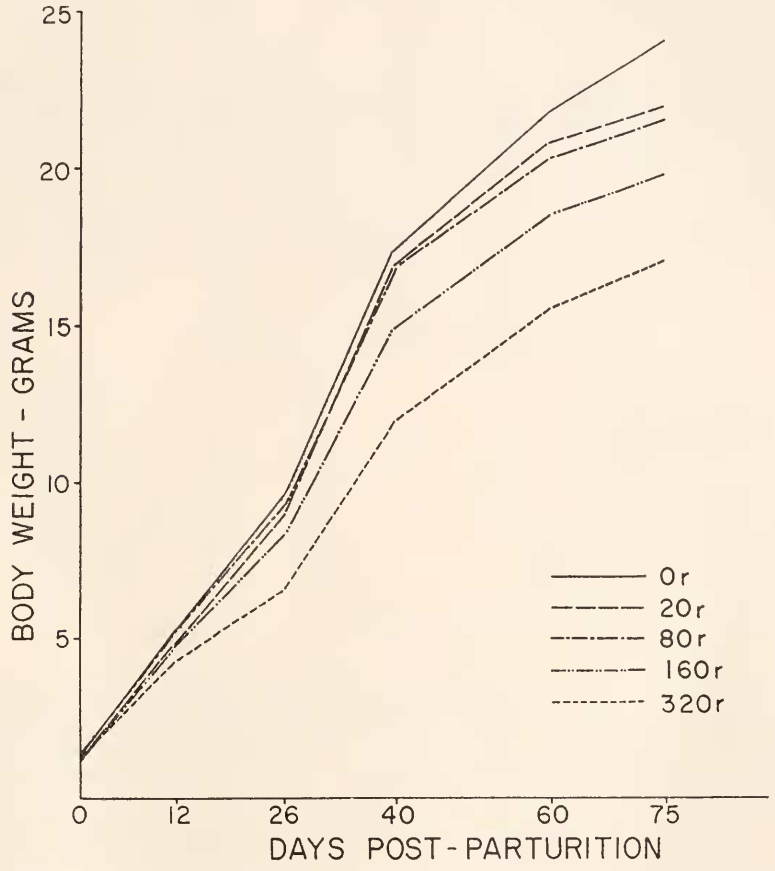

FIGURE 2. Irradiation of newborn mice. Unweighted means of male and female mean weights for each level of irradiation.

Radiation response of females

The mean body weights of the female progeny are shown in Table II. Examination of these data shows that the general body weight response of the females to embryonic irradiation was similar to that of the males. At 75 days of age the same treatments that produced significantly lower body weights in the males have produced significantly lower body weights in the females. These treatments were 80 r at 10½ days, 160 r at 14½ and 17½ days, and 320 r at 14½ and 17½ days. It is of interest to examine these treatments more closely over the period from birth to 75 days. For this purpose the ratio of the Mean Treated Weights/Mean Control

Weights has been calculated for each of these treatments by using the unweighted mean of the male and female mean weights. The results are presented in Figure 1. In general, those progeny that show a postnatal growth depression do not show the maximum response to irradiation until 40 days or more after birth. In addition, there appears to be little recovery by 75 days.

Irradiation of newborn mice

The experiment in which newborn litters were irradiated was begun almost one year after the start of the *in utero* experiment and includes its own group of untreated or control litters. The body weights in this experiment were also adjusted for litter size at birth. The birth weights in this case represent weights taken before treatment. The body weight means and standard errors for male and female progeny are given in Tables I and II, respectively, and the unweighted mean of the male and female weights illustrated in Figure 2. The general response of animals

TABLE III

Breakdown for the statistical analysis

Source of variation	d.f.	Components of variation
Among genotypes	8	$E + 2_j G$
Among dosages	(j-1)*	$E + 18 T$
Genotype × dosage	8(j-1)	$E + 2 GT$
Between sexes	1	$E + 9_j F$
Sex × genotype	8	$E + j FG$
Sex × dosage	(j-1)	$E + 9 FT$
Sex × genotype × dosage	8(j-1)	$E + F G T$
Unaccounted for variation		E

* j = 3, 4, or 5, depending on which embryological age is analyzed.

irradiated at birth closely follows that of animals irradiated as 17½-day embryos. Progeny that were exposed to 320 r are already 20% lighter than controls by 12 days, and, after dropping to 31% at 26 and 40 days, are still 26% lighter at 75 days. Mice given 160 r at birth are significantly lower than controls by 26 days. There is some evidence that newborn litters are more affected by irradiation than 17½ day embryos, as demonstrated by the fact that after a dose of 80 r to newborn progeny, a significant weight change was observed by 60 days of age and was continued through 75 days.

Estimation of components of variation

The amount of variation in body weight response to *in utero* irradiation can be partitioned additively into the amounts due to the various effects and their interactions by utilizing the estimated components of variance derived from an analysis of variance. The general mathematical model upon which the component analysis is based is:

$Y_{ijkl} = u + g_i + t_j + (gt)_{ij} + f_k + (fg)_{ik} + (ft)_{jk} + (fgt)_{ijk} + e_{ijkl}$ where $u =$ the overall mean; $i = 1, 2, \ldots, 9$, the inheritance types; $j = 1, 2, 3,$ (or 4 or 5), the levels of irradiation; and $k = 1, 2$, the sexes. As there were disproportionate sub-class numbers, the method of unweighted means was used in the analysis of

TABLE IV

Components of variance for radiation treatments at different days of gestation in mice as measured by body weights at different days following birth

Components of variance effect	Day of treatment	Birth	Postpartum growth stages					Means
			12	26	40	60	75	
Heredity	$6\frac{1}{2}$	23.1	16.2	15.8	21.8	7.5	4.2	14.8
	$10\frac{1}{2}$	0.9	30.2	28.6	33.9	22.1	17.4	22.2
G	$14\frac{1}{2}$	7.7	3.5	18.3	16.1	12.1	9.2	11.2
	$17\frac{1}{2}$	25.6	34.3	49.9	31.0	12.2	11.1	27.4
	birth	10.8	24.3	27.7	27.2	15.8	13.4	19.9
	Means:	13.6	21.7	28.1	26.0	13.9	11.1	19.1
Dose	$6\frac{1}{2}$	3.4	18.1	22.0	13.7	5.4	2.6	10.9
	$10\frac{1}{2}$	90.6	7.1	15.1	17.3	12.9	10.3	25.6
T	$14\frac{1}{2}$	76.9	2.7	23.8	31.7	25.8	25.4	31.1
	$17\frac{1}{2}$	5.9	20.6	15.6	39.9	40.5	43.2	27.6
	birth	.3	18.0	29.0	41.1	36.7	33.0	26.3
	Means:	35.4	13.3	21.1	28.7	24.3	22.9	24.3
Heredity × Dose	$6\frac{1}{2}$	32.1	53.3	52.6	30.8	17.5	7.3	32.3
	$10\frac{1}{2}$	6.1	49.0	48.1	14.9	9.6	6.9	22.4
GT	$14\frac{1}{2}$	7.4	64.7	43.8	21.9	10.0	8.4	26.0
	$17\frac{1}{2}$	42.4	32.1	27.8	12.4	10.1	5.1	21.7
	birth	51.6	43.5	32.8	13.1	5.3	5.4	25.3
	Means:	27.9	48.5	41.0	18.6	10.5	6.6	25.5
Sex	$6\frac{1}{2}$	15.0	1.7	1.1	22.8	60.2	78.2	29.8
	$10\frac{1}{2}$	.6	0	.4	22.8	49.1	58.9	22.0
F	$14\frac{1}{2}$	1.5	4.4	0.3	12.0	38.0	44.0	16.7
	$17\frac{1}{2}$	9.5	.2	.4	7.8	29.3	35.0	13.7
	birth	6.8	0	.1	8.5	34.5	42.4	15.4
	Means:	6.7	1.3	.5	14.8	42.2	51.7	19.5
Heredity × Sex	$6\frac{1}{2}$	7.2	1.2	1.4	0.9	3.0	1.2	2.5
	$10\frac{1}{2}$	0	0	0	0	0	0.9	.2
	$14\frac{1}{2}$	0	0	0	1.5	3.2	2.8	1.3
GF	$17\frac{1}{2}$	0	0	.2	2.0	.5	0	.5
	birth	0	0	0	.3	.8	0	.2
	Means:	1.4	.2	.3	.9	1.5	1.0	.9
Dose × Sex	$6\frac{1}{2}$	6.0	0	0.5	0.4	0	1.0	1.3
	$10\frac{1}{2}$	.1	0	0	0	0	0	0
TF	$14\frac{1}{2}$	1.0	0	.1	1.7	1.7	1.5	1.0
	$17\frac{1}{2}$	0	0	0	0	1.7	1.1	.5
	birth	0	.6	0	.3	.7	.8	.4
	Means:	1.4	.1	.1	.5	.8	.9	.6
Heredity × Dose × Sex GTF	$6\frac{1}{2}$	0	0	0	0	0	0.1	.0
	$10\frac{1}{2}$	0	0	0	0	.4	0	.1
	$14\frac{1}{2}$	0	0	3.9	7.0	4.7	4.3	3.3
	$17\frac{1}{2}$	0	0	0	1.5	2.3	1.1	.8
	birth	0	0	0	2.3	2.5	1.6	1.1
	Means:	0	0	.8	2.2	2.0	1.4	1.1

TABLE IV—(*Continued*)

Components of variance effect	Day of treatment	Birth	Postpartum growth stages					Means
			12	26	40	60	75	
Random	$6\frac{1}{2}$	12.3	9.5	6.6	9.6	6.4	5.4	8.3
	$10\frac{1}{2}$	1.7	13.7	7.8	11.1	5.9	5.6	7.6
	$14\frac{1}{2}$	5.5	24.7	9.8	8.1	4.5	4.4	9.5
E	$17\frac{1}{2}$	16.6	12.8	6.1	5.4	3.4	3.4	8.0
	birth	30.5	13.6	10.4	7.2	3.7	3.4	11.5
	Means:	13.3	14.9	8.1	8.3	4.8	4.4	9.0

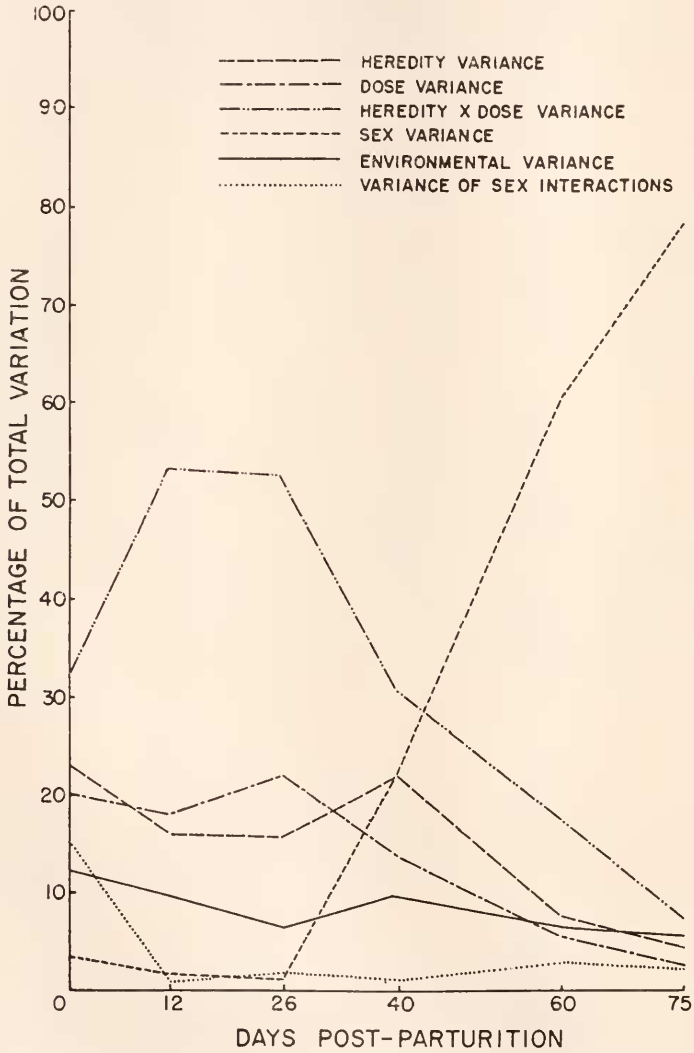

FIGURE 3. Irradiation at $6\frac{1}{2}$ days. Breakdown of variation in body weight. Components expressed as a percentage of total variation.

variance. The general breakdown for the analysis is given in Table III. The components can be interpreted as follows: G is the variation due to genotypic or hereditary differences, T is the variation due to differences in effects of the dosage levels, and F is the variation between sexes. The interaction terms are interpreted

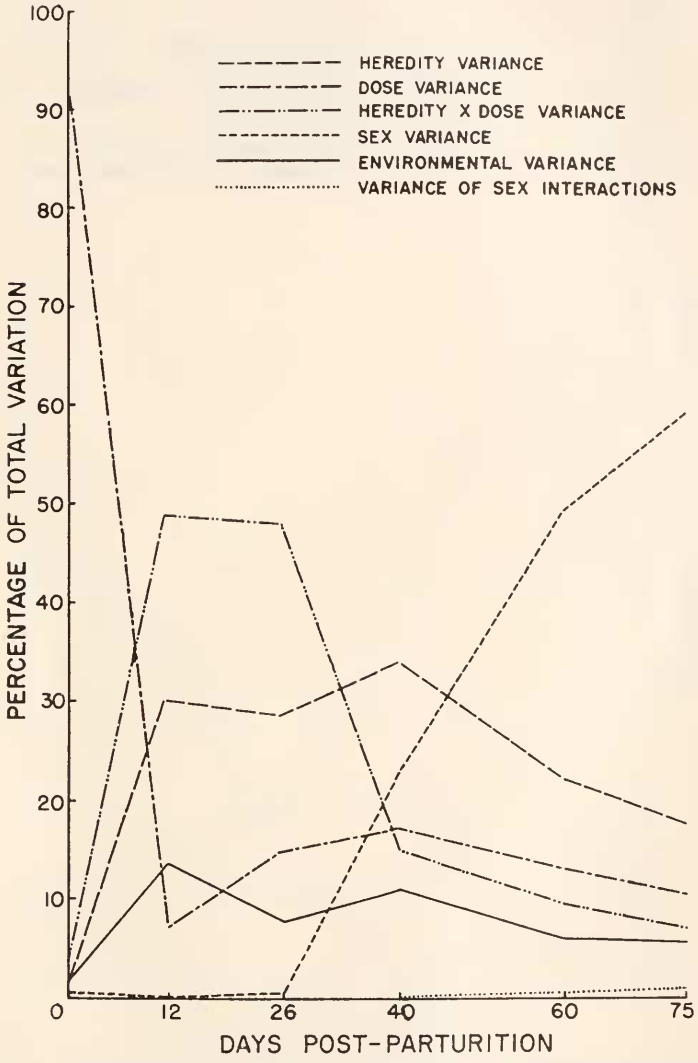

FIGURE 4. Irradiation at 10½ days. Breakdown of variation in body weights. Components expressed as a percentage of total variation.

as arising from the differential responses of the genotypes or sexes from one level of irradiation to the next. The term, E, is considered due to uncontrollable variation, and represents random variation of individual differences of mice of the same sex within a litter given the same treatment. An analysis of variance was obtained

for each of the embryological ages separately. The results of the component analysis are shown in Figures 3–7.

Although there is considerable variation among the different embryological ages in the percentages of total variation that are attributable to the various factors

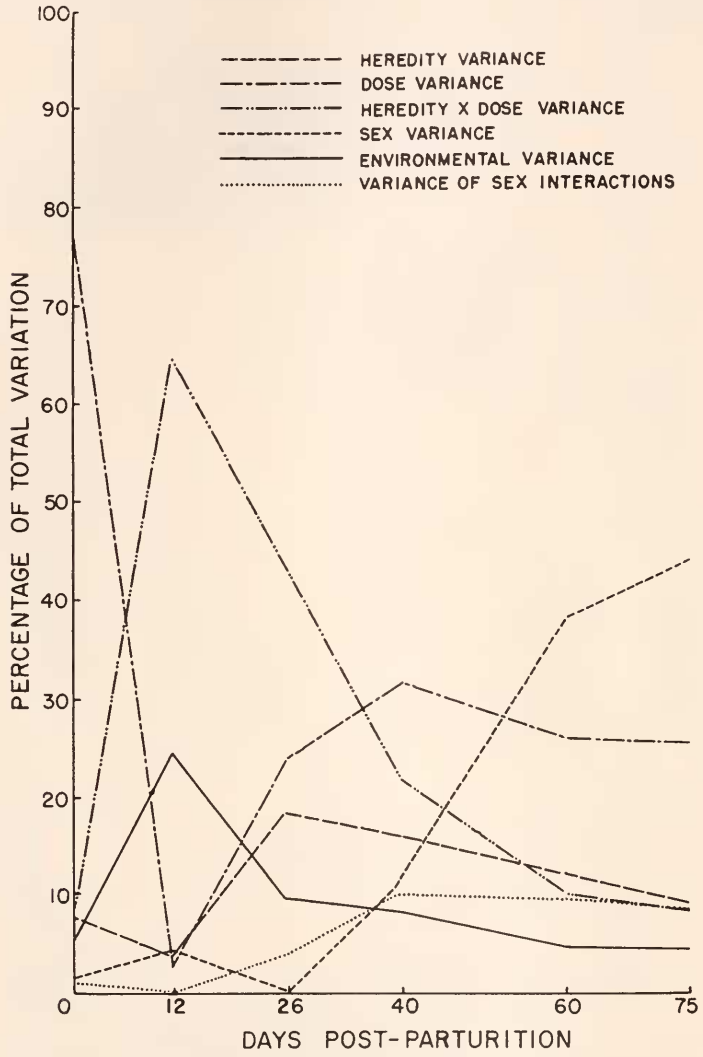

FIGURE 5. Irradiation at 14½ days. Breakdown of variation in body weights. Components expressed as a percentage of total variation.

operative in this experiment, there are some general patterns that hold true for all ages. Within those embryological ages where progeny show the greatest body weight response, not including birth weight response, to irradiation the effects of irradiation do not reach their maximum contribution to total variation until usually

40 days or more after birth. Furthermore, this effect declines little, if at all, by 75 days.

The heredity influences on variance (Table IV) contributed about 20% of the variances for the different growth stages as separated by the x-ray treatments at

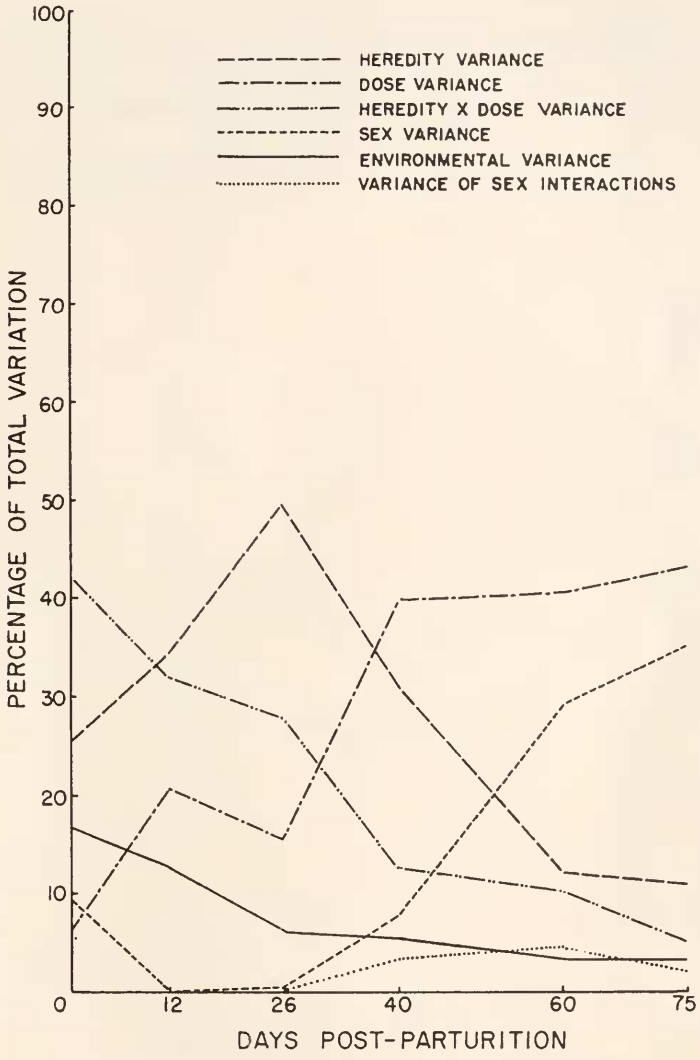

FIGURE 6. Irradiation at 17½ days. Breakdown of variation in body weight. Components expressed as a percentage of total variation.

the specified embryonic stages of development. This average hereditary effect was almost identical with that observed for the mice treated directly after birth, 19.9%. The highest genotypic effects were observed for the 10½- and 17½-embryonic day treatments. Lower values were observed for the embryos exposed at 14½ and 6½

days after fertilization. The hereditary effects were strongest at the 12-, 26-, and 40-day growth periods. The decrease from 40 to 60 and 60 to 75 days post partum corresponded to the point in development where the infant mice were changing from their dependence on both their own and their mother's inheritances (in terms of

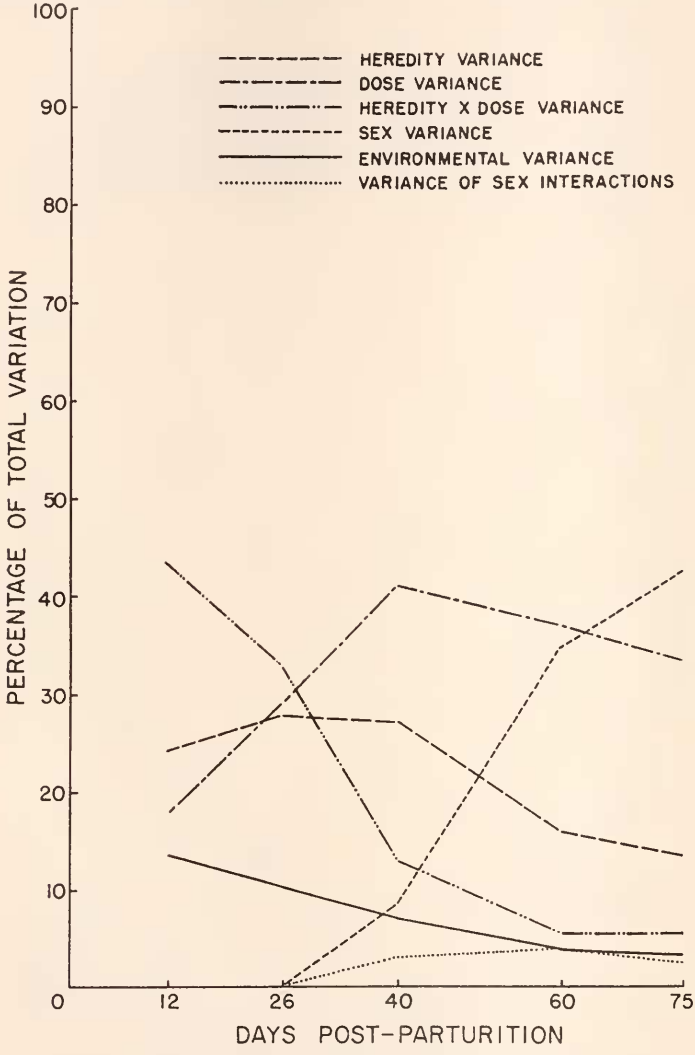

FIGURE 7. Irradiation of newborn animals. Breakdown of variation in body weight. Components expressed as a percentage of total variation.

food, milk supply) to that where their own genotypes were alone responsible for their growths. Both the day of fetal treatment effects and the time when the growth stages were measured appear to show consistent differences in the hereditary effects at about 0.01 significance (Table V). The interaction between fetal

day of treatment and the ultimate effect of the hereditary component on growth at the later period was almost significant at the 0.05 level, showing that there may be some specificity in the mode of action of the hereditary factors.

TABLE V

Analyses of variance of the components for the radiation treatments at different days of gestation as measured by body weights at different days following births

	Source of variation	d/f	M.S.
Heredity	Total	29	
	Day (D)	4	240.2
	Growth (G)	5	255.9
	D × G	20	60.9
Dose	Total	29	
	Day	4	364.2
	Growth	5	276.6
	D × G	20	473.2
Heredity × Dose	Total	29	
	Day	4	105.5
	Growth	5	1405.7
	D × G	20	130.2
Sex	Total	29	
	Day	4	256.9
	Growth	5	2434.9
	D × G	20	57.3
Sex × Heredity	Total	29	
	Day	4	5.9
	Growth	5	1.4
	D × G	20	1.9
Sex × Dose	Total	29	
	Day	4	1.6
	Growth	5	1.3
	D × G	20	1.4
Sex × Dose × Heredity	Total	29	
	Day	4	10.8
	Growth	5	4.5
	D × G	20	1.4
Random	Total	29	
	Day	4	14.7
	Growth	5	92.9
	D × G	20	31.1

The dosage of irradiation components accounted for about 25% of the average variance observed in postpartum growth. Radiation dose appeared most effective for the 14½-day and 17½-day periods. It is to be remembered, however, that the 320 r dose on the 6½-day embryos gave no progeny at birth. The effect of dose

consequently was more severe at the 6½-day stage than appears from the calculated component analysis. The dose components over the different postpartum growth stages showed high variation among the different fetal treatment times. Much of the variation came at birth. Dosage effects appeared low for the 6½-day treatment. The effects were very high for the 10½- and adjoining 14½-day embryos. The nearly formed mice at 17½ days and the mice at birth were not appreciably affected. The dosage effects later in the growth period were high save for the 6½-day, and to a lesser extent for the 10½-day, fetal exposures where deaths in the 16 r and 320 r mice had been extreme. This lowering of the effects could be expected since the treatments already had removed the mice that would show extreme radiation effects, leaving in the later populations only those with lesser damage to have their growths measured. The effects of fetal day of treatment, growth stages when growth measurements were made, and their interaction, had about equal effects as judged by the component variations (Table V). They were probably all highly significant as compared with the uncontrolled variations representing the random element.

The interaction components between the hereditary elements and dosage on the particular days of fetal treatment accounted for about 25% of the variations. For data of this type this percentage was high. High specific effects between the inheritance and the ability of the fetuses to resist the x-ray irradiations at the different fetal ages are indicated. The interaction component values were comparable for the different days when the fetuses were treated. They were most significant in the early growth weights when the mice were nursing. In this period both the heredity differences of the fetuses and of their mothers were expressed. After weaning, a lowering of the interactions of heredity and dosage, as measured by the mouse weights, took place as the mouse became dependent on its own genotype.

The sex components showed variations, as would be expected, with regard to the period postpartum when they were measured. The sex effects on the weights did not become significant until the mice were aged 40 days. This is the period when puberty commences and any differential hormone secretions begin having their effects. The sex differences accounted for 40 to 50% of the variance in the weights at the 60- to 75-day periods when the chromosomal controlled sex differences were well along in their development. The variance among the components for day of treatment and sex effects had a significance of about 0.01 (Table V). The variance engendered by the differences in the growth periods when the components were measured was highly significant. The interaction between day of treatment and growth period was not significant.

The interactions of the components sex by heredity and sex by dose, and the triple interaction sex by dose by heredity, contributed but little to the total variance. Although they showed some evidence of unequal effects for day of fetal treatment they will not be discussed because of the small size of these effects.

The random or uncontrolled variances averaged about 9.0% of the total variance, a surprisingly small value. The components for this random variation are rather consistent for the days when the fetuses were treated. They show significant decreases in value from the birth to the 75-day postpartum weights. These changes may in part be due to the large variations that may be induced by the differences in quantity of milk contained in the stomachs of the just-born mice as contrasted with mice of older ages.

The variance analyses of the component values shown in Table IV are presented in Table V.

Discussion

The observations herein presented confirm as well as extend the conclusions that may be derived from the results of some of the earlier investigations in this field. Job *et al.* (1935) were the first workers to demonstrate clearly the presence of well-defined critical embryological periods susceptible to x-ray irradiations, as measured by morphological abnormalities, although earlier workers such as Von Hippel and Pagenstecher (1907) and Kosaka (1927, 1928) observed malformations following embryonic irradiations. Job *et al.* found that pregnant rats irradiated with 35 to 90 r between the eighth and eleventh day of gestation delivered abnormal embryos. The critical periods established for certain defects appeared to be: hydrocephaly, ninth day; eye defects, tenth day; and jaw abnormalities, eleventh day.

Russell (1950, 1956) studied gross visceral and skeletal malformations induced in mouse embryos following irradiation with doses of 100 r to 400 r between ½ and 13½ days of gestation. She found a wide variety of abnormalities including microphthalmia, polydactyly, limb deformities, coloboma, vaulted cranium, spina bifida, imperforate anus, tail abnormalities, hydronephrosis, and open eyelids. Using the criteria of prenatal mortality and abnormalities at birth, Russell found that the prenatal development of the mouse was divisible into three broad phases:

The pre-implantation period (½–4½ days). Irradiation during this period with x-ray doses of 100 r–200 r gave a high incidence of prenatal death, but virtually no abnormalities among those embryos surviving to term.

The period of major organogenesis (5½–13½ days). Irradiation during this period with doses up to 400 r gave almost no prenatal loss of embryos, but did cause a high incidence of malformations at birth.

The period of the fetus (14½ days to birth). Irradiation during this period of growth and minor organogenesis did not cause prenatal deaths nor any gross abnormalities at birth, although several types of abnormalities have been observed to occur later in life.

There have been few quantitative experiments concerned with the effects of embryonic irradiation upon postnatal growth. Cohn (1907) and Hanson (1923) reported reduced body size in adult stages in the rabbit and rat, respectively, but details as to levels of irradiation and age of embryos when irradiated are lacking.

Russell (1950) in an extensive experiment found that the mean birth weights of mice that had been irradiated between 8½ and 13½ days were considerably lower than those of the controls. The most critical period for both 200 r and 300 r appeared to be between the 10½- and 11½-day stages. The mean weight at 11½ days for the 200 r dose was only two-thirds of that of the controls. The work on the 11½-day stage was extended by Russell, Russell and Major (1951), and showed that points for different doses and for the controls fell on an approximately straight line, with weight reduction per 100 r averaging 0.22 gram over the three available intervals.

Wilson *et al.* (1953) found that seven embryos that had received 100 r on day 10 weighed on the average 10% less than controls at birth, but recovered this initial weight deficiency by 50 or 60 days postpartum. Ershoff and Bavetta (1958),

however, observed no difference in average weights of rats at birth or at 21 days following irradiation with 150 r on days 10 or 14. Graham *et al.* (1959), using the same treatments and the same strain of rats, did find a 10% weight decrease at birth, but surviving rats were "normal" at weaning.

Levy *et al.* (1953) irradiated mouse embryos of 15½ days with 300 r, and examined the femur, mandible, and parietal bones at birth and various other days to 240 days of age. Irradiated embryos were born with bones having dimensions smaller than normal, and there were significant differences between averages on various days for both control and irradiated animals for all measurements between one and 29 days postpartum. In general, irradiated animals maintained smaller bone dimensions compared to unirradiated, although differences were not as marked as time went on.

The sampling of the entire gestational period by observing effects of x-irradiation given at four different embryological ages, namely, 6½, 10½, 14½, and 17½ days, in the present study did indicate "critical periods" for the induction of changes in postnatal growth. These critical periods are in agreement with those using body weight at birth as a criterion, as found by Russell (1950), who observed that day 11½ was close to the stage of maximum susceptibility for growth retardation. The embryological ages in order of increasing response to birth weight depression in the present study were found to be 6½, 17½, 14½, and 10½ days. The same order of sensitivity was shown in postnatal growth, if allowance is made for the fact that no progeny at all survived irradiation with 160 r or 320 r at 10½ days. This stage was the only stage in which a dose of 80 r had an effect on postnatal growth, lowered body weights having been observed by 60 days postpartum after irradiation with 80 r at 10½ days whereas 80 r at other ages did not affect body weight.

In evaluating effects of *in utero* irradiation it is necessary to consider the possible role of the maternal organism in producing abnormal development in the embryo. The evidence of various workers, including Russell (1950), Hicks (1950), Wilson and Karr (1951), Brent (1957), and Grayevsky *et al.* (1959), indicates that the role of the maternal organism is of no consequence or of very little consequence in producing morphological abnormalities observable at birth. The influence of the irradiated maternal organism on postnatal growth has not been explored adequately. Russell (1950) found that mortality after birth was apparently not due to inability of mothers that had been exposed to whole body irradiation to care for the progeny, since irradiated females were able to raise young mice to weaning age when they were given non-irradiated newborn litters to foster. No mention was made of body weights of these foster litters.

Rugh (1956) studied effects on growth of suckling young from irradiation of lactating mothers. Female mice were irradiated two days after delivery of litters. An increased retardation of growth was found in the young. There was considerable variation in results between separate experiments, making it difficult to generalize the quantitative effects of maternal irradiation on the growth of young.

Neither of the above examples provides information on direct effects of *in utero* irradiation upon postnatal growth and the indirect effects through changes in lactating ability of the mother when both of these factors are operative at the same time. The following points in the present experiment provide some indirect evidence that indicates most of the effects of postnatal growth are due to direct effects on the irradiated embryo.

Irradiation of newborn progeny without the maternal organism receiving any irradiation at all still resulted in severe disturbances in postnatal growth. A dose of 320 r given to young at birth produced body weight changes which were similar in magnitude to those obtained after a dose of 320 r at 17½ days gestation.

After irradiation with 20 r or 80 r at any of the embryological ages, and 160 r at 6½ days, postnatal growth was normal, indicating that these dose-embryological age combinations were ineffective in affecting the subsequent lactation of the mother.

In those treatments that yielded significantly lower body weights the maximum effect was not reached until weaning or later, and there was little recovery even by 2½ months of age. It appears from these results that during the early phases of postnatal growth, radiation-induced damage in the young is compensated somewhat by the nutrition furnished by the maternal organism. However, as the young shift more towards solid food and are weaned, they become completely dependent upon their own physiological systems. It is then that the direct effects of radiation may become most noticeable.

It appears for these reasons that the effects of *in utero* irradiation upon postnatal growth are due, for the most part, to direct effects of the radiation upon the embryo, and that irradiation of pregnant females has little effect on the lactation of these females. The possibility should not be excluded, however, that some specific dose-embryological age combination may have an effect on lactation which would be reflected in the postnatal growth of the young.

It is important in evaluating effects of *in utero* irradiation to consider the time at which observations are made. Thus, in some of the treatments which affected postnatal growth the most, it was not yet apparent by birth or even 12 days that growth would be retarded. It is evident in these cases that there had not been sufficient time for this type of radiation damage to have been expressed. An assessment of radiation-induced changes should always specify the criteria that are being used to determine damage.

It is of some interest to examine the similarities between these radiation-induced growth changes and growth changes effected by mutant genes, although as Russell (1954) has emphasized, it is unlikely that gene action should parallel exactly the pattern of radiation response. The two best known mutants which affect growth in the mouse are pituitary dwarfism and pygmy. There are some striking dissimilarities between effects of these genes. Pituitary dwarfism, originally described by Snell (1929), causes practical cessation of growth at 14 days postpartum. The primary effect of the gene appears to be on the anterior lobe of the pituitary.

Pygmy, first described by King (1950), is apparently not due to a lack of growth hormone, and reduction is already manifest by birth. King concluded that it is possible that the effect of this mutant is to reduce the responsiveness of tissues of the body to the growth component of pituitary hormone.

Additional types of dwarfism in the mouse include a type described by Strong (1948) which is apparently different from both pituitary dwarfism and pygmy in that affected animals are not only small at birth but also exhibit restlessness. More recently Schaible and Gowen (1961) have described a new dwarf mouse, which, although it is phenotypically similar to the pituitary dwarf, has been found not to be allelic.

It is evident from these examples that the mutant forms of dwarfism have a wide

variation in the way they affect growth. In the present experiment in which entire embryos were exposed to irradiation, it appears likely that in those treatments in which weight depression was observed by birth, the result has been due to effects of various cells throughout the body which produce metabolic derangements interfering with normal assimilation and growth, and/or to effects on the pituitary gland or other growth-controlling organs which could have affected growth.

In those treatments with which there was a considerable growth retardation some time after parturition, the general growth curve and response were somewhat similar to that produced by pituitary dwarfs, although this does not mean that the pituitary gland may have been affected to some degree. The channels through which growth may be regulated are numerous, and some of the growth retardation probably is also due to direct effects of radiation on other tissues, as well as to effects on the pituitary and/or a number of other secretory glands. It will be seen in a subsequent paper that some of the treatments produced progeny which were not only stunted, but also had an increased mortality rate throughout life, a higher incidence of cataract formation, and decreased fertility.

It was seen earlier that there were considerable genetic differences in susceptibility to radiation-induced growth changes. It is likely that these differences in response are a result, in part, of differences in developmental rates of the various genotypes. Even a small difference in developmental ages during a time of rapid differentiation at the time of radiation exposure could result in a large difference in subsequent development. In addition, body weight response may be influenced by genetic variation in response to the secondary effects of radiation. Grahn (1954) determined body weight changes in six inbred strains of mice irradiated at 40 days of age, and observed clearcut genetic differences. His study is of particular importance to the present investigation since it included the Ba and S strains used in the present study. Grahn found that there were not only differences in the maximum body weight loss following radiation exposure, but that there were also genetic differences in the rate, time, and completeness of recovery from radiation effects. Comparisons between a susceptible (Ba) and resistant (S) strain indicated strain differences even after a low dose of 20 r. All of the strains showed a similar dose-response curve, indicating that the basic response was similar in the six strains. Genetic differences were being expressed through the rate of recovery from disturbed physiological activities. Due to the overall size of the present experiment the number of observations for any single genotype for any one treatment was of necessity small, and precludes the making of meaningful comparisons of individual genotypes. Nevertheless, it appears that the genetic differences between strains are influencing the different radiation effects on growth following irradiation of embryos. These genetic differences may be exerted through the abilities of individual cells and organs to resist the deterimental effects induced by radiation. Mice of certain genotypes may be able to repair damage and return to normal physiological activity more quickly than mice of other genotypes.

Summary

1. The effect of x-irradiation of mouse embryos upon their postnatal development was measured by several responses: body weight changes from birth to maturity, lifetime fecundity, and total lifespan. The body weight responses are re-

ported in this paper. Three genetically differentiated inbred strains of mice, Ba, K, and S, and all their possible hybrids, including reciprocals, were used. Pregnant females were exposed to single whole-body 250 pkv x-ray dosages from 20 r to 320 r on $6\frac{1}{2}$, $10\frac{1}{2}$, $14\frac{1}{2}$, and $17\frac{1}{2}$ days gestation, as timed from the appearance of a vaginal plug. In addition the study included progeny irradiated on the day of birth without any irradiation of the maternal organism. Postnatal growth was followed from birth to 75 days of age, individuals having been weighed at birth, 12, 26, 40, 60 and 75 days.

2. Body weights were adjusted by making use of the pooled regression coefficient of body weight on litter size over all treatments. Body weight response was found to be dependent on both level of irradiation and embryological age at irradiation. The embryological ages in order of increasing sensitivity were $6\frac{1}{2}$, $17\frac{1}{2}$, $14\frac{1}{2}$ and $10\frac{1}{2}$ days. Body weight response was found also to be markedly dependent upon the age at which observations were recorded. In those treatments that produced significantly lowered body weights the maximum effect was not found usually until 40 days postpartum. Growth effects appeared to be permanent since there was little recovery evident by 75 days. Evaluation of these results emphasizes the importance of considering both immediate and delayed effects in assessing damage induced by embryonic irradiation.

3. Growth differences following embryonic irradiation were found to be under a strong genetic influence. Genetic differences in response to the induction of growth retardation were thought to be expressed as a result of genetically determined differences in recovery from disturbed physiological activities and differences in developmental age of embryos at the time of irradiation.

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